

## REVIEW ARTICLE

# CROATIAN SOCIETY OF CLINICAL EMBRYOLOGISTS – GUIDELINES ON THE EPIDEMIOLOGICAL FRAMEWORK FOR THE IMPLEMENTATION OF MEDICALLY ASSISTED REPRODUCTION (MAR) PROCEDURES DURING THE COVID-19 PANDEMIC REGARDING THE SAFETY OF PATIENTS AND MEDICAL HEALTH WORKERS

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**Abstract:** Due to the high virulence of the SARS-CoV-2 virus, the infection rate in the community has led to a state of pandemic, leading to the introduction of new emergency measures all over the world. With the aim of controlling and preventing the SARS-CoV-2 viral epidemic, the health institutions performing medically assisted reproduction (MAR) suspended any new MAR treatments in order to reduce the burden on the health care system and implement current social distancing recommendations. Considering the favorable epidemiological situation in Croatia, our perspective is that it is time to conceive, plan and bring forth guidelines for restarting work in MAR centres, taking into account the selection of patients and organization of good laboratory and clinical practices with emphasis on the safety of patients and health workers. In regard to epidemiological knowledge, it is important to establish the reorganization of work in MAR centres including epidemiological measures of reducing unnecessary stays in closed spaces, the usage of protective gear by patients and health workers and disinfection of the working spaces and equipment.

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## INTRODUCTION

The world is facing a new viral pandemic, Coronavirus Disease 2019 (COVID-19), that has spread quickly owing to its rapid community transmission, high virulence and sustained surface viability. Many infected and contagious individuals may only have mild symptoms, including fever, or, more rarely, they may be asymptomatic. COVID-19 is a respiratory illness caused by the novel coronavirus Severe Acute Respiratory Syndrome SARS-CoV-2, a single-stranded RNA virus. The term “COVID-19” is used to represent the viral respiratory disease and “coronavirus” to represent the SARS-CoV-2 virus.<sup>1</sup>

Living and operating in a society where COVID-19 exists is becoming a reality for many. Data suggest that COVID-19 will remain a factor to be managed in our lives and practices for a prolonged period of time. The ultimate response to this pandemic may rely on the development of a vaccine that prevents COVID-19, or of effective treatments, or both.

As the COVID-19 pandemic is stabilizing, the return to normal daily life will also see the need to restart the provision of medically assisted reproduction (MAR) treatments.<sup>2</sup> Infertility is a disease, and once the risk of SARS-CoV-2/COVID-19 infection has decreased, all assisted reproductive technology (ART) treatments can be restarted for any clinical indication. However, vigilance and measured steps must be taken for safe practice and to minimize the risks related to SARS-CoV-2/COVID-19-positive patients or staff during treatment.

## DISCUSSION

### *Croatian Society of Clinical Embryologists consensus regarding IVF laboratory work and safety*

To avoid the consequences of SARS-CoV-2/COVID-19 infection on IVF laboratory work, at a meeting on 27th April 2020, Croatian Society of Clinical Embryology members considered the following topics and reached the following consensus:

#### **COVID-19 testing**

Two main methods are used for the detection of virus infection: molecular (direct) and serological (indirect) method.<sup>3</sup> The molecular method is based on the isolation of SARS-CoV-2 RNA from a nasopharyngeal swab and throat swab and the amplification of a few virus-specific genes (2-4) with RT-qPCR. While it is an expensive and time-consuming method, RT-qPCR tests are highly specific with low probability of false positives, but false negatives can occur if the sample contains insufficient quantities of the virus for successful amplification and detection. The amount of virus in a swab is likely to vary between patients, sample location (nasal, throat or sputum) and over time as the infection progresses.

Instead of detecting viral genetic material, serological tests target the immune response of the infected person, looking out specifically for antibodies against the virus.<sup>4</sup> These tests are cheap and fast. The problem with these tests is that antibodies only develop several weeks after an infection, which means that antibody-based tests might miss asymptomatic cases or people in the earliest stage of the disease. Most serological tests are in the research stage of development and need further validation to determine their accuracy and reliability.

As RT-qPCR looks for viral RNA, it will give a positive test result only if there is an ongoing infection. On the other hand, antibodies can persist for months or years, allowing tests to identify anyone who has ever been infected.

#### **Disinfection and disinfectants**

In order to protect patients, their reproductive cells, embryos and embryologists who treat them, it is important to follow the basic disinfection and protection steps before entering the lab, in the lab, and after leaving the lab:

1. Hand washing before putting on protective equipment.
2. Disinfection of work surfaces before work.
3. Disinfection of work surfaces between each patient.
4. Disinfection of work surfaces and work incubators at the end of a working day.

5. Disinfection of all items brought in and out of the lab.
6. All paper required in the lab should be in easy-to-disinfect plastic liners.
7. Disinfection of incoming media vials (before first refrigerating and after each media preparation) and packaging of consumables before entering into the laboratory.
8. Regular disinfection of incubators, cryo buckets, tweezers, pipettors, markers etc.

Disinfectants with proven anti-enveloped viral activity should be used exactly according to the instructions for use: contact time, dilution and shelf life of concentrates and diluted solutions.

Comparison of the genetic characteristics of SARS-CoV-2 and Middle East respiratory syndrome coronavirus (MERS-CoV) suggests that SARS-CoV-2 is susceptible to active compounds in disinfectants that have been shown to be active against other enveloped viruses.<sup>5</sup> Effective disinfectants are those which have any of the following active compounds: sodium hypochlorite, bleach (0.1% -1%), ethanol (62-71%), hydrogen peroxide, phenolic compounds, 5% chlorinated agents, quaternary ammonium compounds. For disinfection of surfaces and incubators in the IVF laboratory, it is possible to use only disinfectants which have the quaternary ammonium compounds as active substance, such as Oosafe product lines.

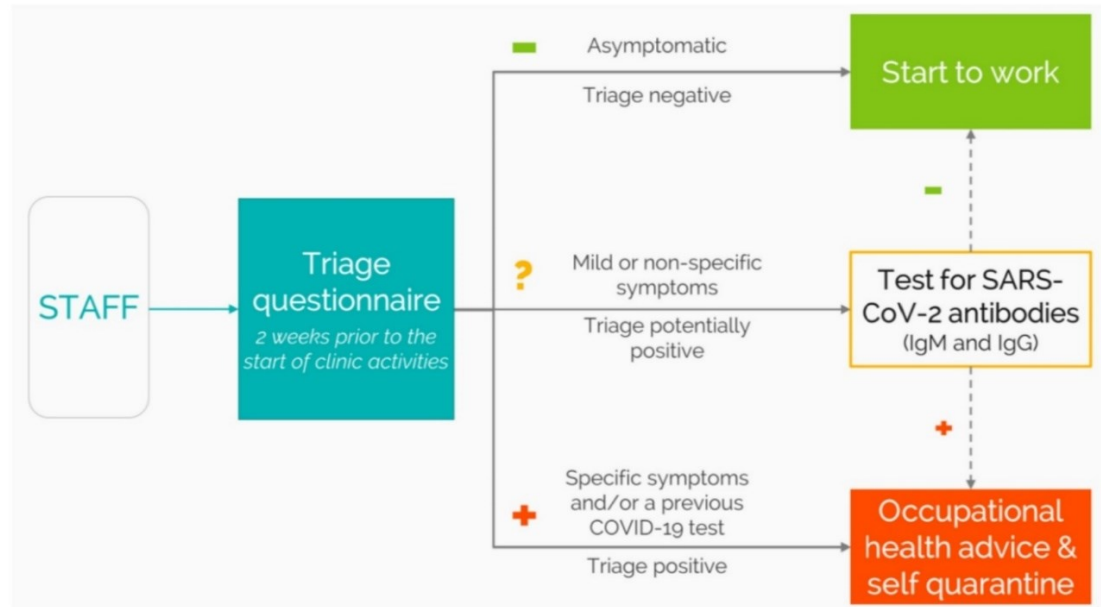
Air and space disinfection is possible with ultraviolet light (UV-C spectrum, about 250 nm).<sup>6</sup> Ultraviolet radiation inactivates the nucleic acid of the virus (in this case ssRNA, which is the most susceptible to the UV radiation of all nucleic acids). The energy required for inactivation of the viral ssRNA is 1.32-3.20 mJ/cm<sup>2</sup> with lower energy required when the room humidity is lower. If the MAR centre decides to use UV disinfection of air and space, the previous chemical surface disinfection as well as the necessary precautions and additional training for the employees should be taken into account.

#### **Procedure for MAR laboratory staff members**

Triage questionnaire regarding health status, symptoms and lifestyle of all clinic team members two weeks prior to the start of all MAR laboratory activities (Figure 1), and the ART triage questionnaire (Appendix 1) for staff and patients when making appointments by e-mail or telephone should be applied.<sup>2</sup>

Subdivision of the laboratory staff into mini-teams (1 embryologist + 1 laboratory technician or 2 technicians in the case of more andrology procedures, such as semen cryopreservation) with minimum interaction among them is recommended.

Mini-teams should work according to a rotating schedule every two weeks or a daily-based shift with the use of all necessary protective clothing (masks, disposable gloves and coats). If the teams split into



**Figure 1. Staff triage (ESHRE guidance on recommending ART treatments, document prepared by the ESHRE COVID-19 Working Group, published on the ESHRE website, date of publication: 23/04/2020)**

daily-based shifts, there should be one hour between the shifts when the laboratory should be cleaned. To follow epidemiological measures, it is important to reduce unnecessary visits and embryologist/laboratory

**Appendix 1. ART triage questionnaire (ESHRE guidance on recommending ART treatments, Document prepared by the ESHRE COVID-19 Working Group, Published on the ESHRE website, Date of publication: 23/04/2020)**

#### ART Triage Questionnaire

1. Have you been sick in the last two weeks?
2. Do you have fever (over 37,5°C)?
3. Are you coughing at present?
4. Do you have a sore throat?
5. Have you lost your sense of smell or taste?
6. Have you been in contact with somebody who has any of these symptoms?
7. Have you travelled to an area at high risk for COVID-19, nationally or internationally?
8. Do you work in a hospital/nursing home or healthcare facility?
9. Have you been in contact with somebody who has COVID-19?
10. Have you been you diagnosed with COVID-19?
11. Do you live in a household with somebody who has been diagnosed with COVID-19 infection or has COVID-19 symptoms (fever, cough, loss of smell)?
12. If you have been COVID-19 positive and recovered, do you have certified medical evidence of clearance?
13. Do you have a severe medical condition like diabetes, respiratory disease, chronic kidney disease, etc.? (this question can be skipped when using the ART triage questionnaire for staff).

technician-patient contact (e-mail or telephone). Also, it is important to restrict access for partners and accompanying persons not needed for procedures, possibly redesign waiting rooms to guarantee appropriate distancing.

Appointments should be managed according to the specific timetables, and the number of patients in the waiting rooms should be limited. Also, there should be limited number of staff members in the transfer room (embryologist, gynecologist, nurse) with the patient.

#### ***SARS-CoV-2 - The presence of virus in semen and testicular tissue***

Considering the evidence that novel SARS-CoV-2 can cause more than the respiratory symptoms characteristic for the coronavirus family, and especially due to its structural similarity to the previous SARS-CoV-1, there are concerns that the infection could also affect the male reproductive system.

SARS-CoV-1 has been associated with defects in spermatogenesis, testicular damage and inflammation, but it is still a matter of debate whether the virus is present in the tissue and whether the damage is caused directly by virus cell/tissue binding or by the immune system reaction.<sup>7</sup> SARS-CoV-2 uses the same receptor angiotensin-converting enzyme 2 (ACE2) for cell entry as SARS-CoV-1. There are several studies about the expression pattern of ACE2 in human testis. Fan et al.<sup>8</sup> used RNA and protein expression data available online and found strong expression of ACE2 in testicular tissue. Furthermore, Wang et al.<sup>9</sup> analyzed ACE2 at single-cell transcriptome level and found that it is

primarily expressed in spermatogonia and Leydig and Sertoli cells. Early spermatocytes, late spermatocytes, spermatids and other somatic cells had very low expression levels of ACE2. The expression of transmembrane serine protease 2 (TMPRSS2) which enables S protein priming on the SARS-CoV-2 envelope was also analyzed. TMPRSS2 expression was concentrated in spermatogonia and spermatids. Additionally, they found that ACE2-positive spermatogonia express a higher number of genes associated with viral reproduction and transmission, and a lower number of genes related to spermatogenesis compared with ACE2-negative spermatogonia. These studies suggest that there is theoretically a potential for viral binding and replication in testicular tissue, which could cause tissue damage, impairment of testicular function and potentially sexual transmission of the virus. It is important to note that there is one study with contradictory results, according to which the expression of ACE2 and TMPRSS2 in testicular tissue is very low, their co-expression is very rare and therefore virus binding and testicular infection is unlikely.<sup>10</sup> There is no evidence of SARS-CoV-2 testis infection so far. Only one tissue sample from patients who died of COVID-19 was tested negative for viral RNA.<sup>11</sup>

Viral RNA has been detected in feces and rarely in blood and urine. The studies published so far did not detect SARS-CoV-2 in semen. A total of 47 semen samples from patients with mainly mild to moderate symptoms have been tested. At the time of testing, 12 patients were in the recovery phase (11 patients with a negative, one patient with a positive swab), 34 patients approximately one month from a positive swab, one patient eight days after a positive swab.<sup>12</sup>

The above-mentioned studies are the first studies of SARS-CoV-2 related to male reproductive system. They have been conducted with certain limitations. Studies did not include larger sample size, patients with more severe symptoms and at an earlier phase of infection. More research is needed to elucidate the impact of the SARS-CoV-2 on the male reproductive system and until then, caution is recommended during semen and testicular tissue processing.

A semen sample should be collected at home and the patients should deliver the sample to the laboratory within one hour of ejaculation in order to minimize their time spent at the fertility centre. If the patients are unable to collect the sample at home or bring it within one hour, the sample may be collected in a collection room that is thoroughly disinfected after each patient. There should be a time gap between patient arrivals at the centre.<sup>13</sup>

The semen sample to be used in the in vitro fertilization treatment should be processed on a density gradient with an additional swim-up step. The addition of the layering culture medium to the pellet from the density gradient is an additional step in the purification of the sample.<sup>14</sup> This kind of the semen preparation of the

HIV positive patients has been shown to remove more than 99% of viral particles from the sample.<sup>15</sup> The samples can be processed by a simple wash technique in the cases where a small number of sperm is present in the sample (severe oligoasthenozoospermia, testicular tissue) as they are not suitable for purification on a density gradient.<sup>16</sup> No cases of viral transmission have been reported in the literature following the use of prepared semen in the IVF treatment.<sup>17</sup>

### ***Follicular aspirate, oocytes and Covid-19 – viral presence and isolation procedure***

Recent research on the topic of oocyte isolation in time of the COVID-19 pandemic is virtually non-existent. It is important to determine the presence of viral SARS-CoV-2 particles in follicular aspirate after oocyte retrieval in order to develop safe protocols for oocyte isolation to protect the staff and resolve issues concerning viral presence in oocyte culture. There are various confounding factors in recent research that can affect the use of results for developing protocols that include: small study samples, sampling patients after recovery from the infection, or age not suitable for reproduction.

Since there is an absence of direct proof of the presence of SARS-CoV-2 particles in the follicular aspirate, an analysis of available literature concerning viral entry pathways in the cells of the female reproductive system has been conducted.

A team of experts have analyzed 10 vaginal swabs from postmenopausal women in China after they recovered from COVID-19, and the swabs were sterile, which led to the conclusion that no viral particles are present in vaginal secretion.<sup>18</sup> It is an important fact for performing an oocyte retrieval as regards the safety of medical staff, and it is also possible to exclude the contamination of follicular fluid with the viral particles via transvaginal ultrasound probe or puncture. Research has shown that the ACE2 is an important cell receptor for SARS-CoV-2 viral particles and, in cooperation with TMPRSS2, which cleaves the S protein from the viral envelope, it enables viral invasion of the cell.

Having that in mind, it was crucial to establish the presence of ACE2 and TMPRSS2 in tissues of human reproductive system. Ectocervix, endometrium and ovaries do express ACE2 receptors in cellular membranes. In addition, ACE2 expression has been confirmed in the entire process of ovarian folliculogenesis, from primordial to antral follicles, and the expression is amplified when under gonadotropin stimulation protocol. ACE2 is indirectly correlated to oocyte in vivo maturation. TMPRSS2 is somewhat less expressed and does not play an important role in the female reproductive system as it does in the male reproductive system, where it is a key factor for prostate cancer development. Despite of the receptor presence, SARS-CoV-2 particles have not been found

in the female reproductive system so far.<sup>18</sup>

Concerning similarities in SARS-CoV-1 and SARS-CoV-2 structure, a comparison was made regarding their presence in the female reproductive organs. A research showed that women deceased from SARS-CoV-1 infection did not have viral particles in their reproductive organs.

An important case study showed that it is possible to conclude that the virus can infect a fetus in utero, but the pathway of the infection is still unknown.<sup>19</sup>

Also, since ACE2 receptors are abundantly present in the vascular endothelium, it is acceptable to presume an increased risk when performing ovarian puncture procedures since follicles are enveloped in a network of fine blood vessels that can possibly be a source of viral contamination for follicular fluid and pose a health risk for medical and laboratory staff. Undoubtedly, regulation of ACE2 is disturbed with gonadotropin stimulation and can, if combined with COVID-19, lead to pathophysiological changes in ovaries, multiplying the risk for patients - by treating infertility their fertility becomes endangered.<sup>20</sup>

Considering all the presented information, it is impossible to be sure of viral presence in follicular aspirate and to assess the risk of being affected with SARS-CoV-2 while performing oocyte isolation. It is imperative that the procedure is done with extra care in applying safety measures for all staff and all patients that are undergoing ART procedures.

European Society for Human Reproduction (ESHRE) guidelines<sup>21</sup> for performing oocyte retrieval recommend standard safety protocols and disinfecting measures and include fast disposal of the follicular fluids in special containers that are removed from the laboratory premises immediately after the procedure. It is also important to separate patient procedures by at least an hour in order to thoroughly clean and disinfect all surfaces and allow for air filtration to remove any potential contaminants of the air.

### ***In vitro fertilization technique, cell culture and COVID-19***

To minimize the risk of SARS-CoV-2/COVID-19 infection during oocyte fertilization, it is recommended that the intracytoplasmic sperm injection (ICSI) technique is applied. At the time of oocyte retrieval, the virus may be in the follicular fluid, and thus may attach to granulosa cells surrounding the oocyte. Before the ICSI procedure, these cells are removed enzymatically and mechanically from the oocyte. Therefore, the ICSI procedure reduces the chances of possible viral infection. Regardless of viral status, it is recommended that these precautions take place for each patient as part of good laboratory practice.<sup>22</sup> Devaux et al.<sup>23</sup> showed in 17 women positive for Hepatitis C Virus (HCV) that viral RNA was detected by PCR in 89% of follicular fluid samples. After denuding the oocytes of granulosa cells, inseminating the oocytes via ICSI, washing the

oocytes and embryos and refreshing the media during culture, the viral load became undetectable, irrespective of the original follicular fluid status. Therefore, the authors concluded that follicular fluid must be considered as potentially infected, but all oocytes can be inseminated by ICSI resulting in virus-free embryos in culture.

To reduce the spread of potential infection in cell culture, the use of individual oocyte/embryo culture under paraffin oil is recommended. The workspace, laminar hood, and inverted microscope stage need to be disinfected (e.g. Oosafe) and cleaned with sterile water after each procedure.

It is recommended that these precautions take place during oocyte/embryo culture.<sup>24</sup>

- Use personal protective equipment (laboratory coat that covers wrists, hair cover, shoe cover, mask, gloves)<sup>21</sup>
- Use micropipette tips with filter
- Turn on laminar hood air flow minimum 10 min before work
- Disinfect the laminar hood working area (e.g. Oosafe), followed by cleaning with sterile water

### ***Cryopreservation of biological samples and SARS-CoV-2***

SARS-CoV-2 is a RNA coronavirus with the ability of surviving low temperatures and reactivation after the stable conditions in culture media are retained.<sup>25</sup> Extreme caution is advised when freezing and thawing the biological samples (semen, testicular tissue, oocytes and embryos) because of possible contamination with viral particles.<sup>26</sup> Since oocytes and embryos do not have the necessary receptors for SARS-CoV-2 to attach to them, there is no risk of viral integration in the host genome, but there is a possible danger of transmission of the virus through the culture media or liquid nitrogen to the patients or medical staff.<sup>27</sup> To lower the risk of virus transmission through contaminated culture media, it is necessary to heavily dilute the samples (oocytes, embryos) before the freezing procedure. Because of the possibility of contamination of liquid nitrogen with the SARS-CoV-2 virus, and thus contamination of biological sample, avoiding any contact of biological sample and liquid nitrogen before the freezing procedure (slow freezing method or vitrification with the closed system) is advised.<sup>28</sup> When freezing the semen samples or testicular tissue, it is necessary to follow the standard protocol with additional handling precautions (there is no direct contact between the sample and liquid nitrogen, because the sample is stored in closed cryogenic straws and cryogenic vials).

Recommendations for handling biological material in the process of freezing/thawing during the SARS-CoV-2 virus are:

- Sterilization of the equipment (forceps, steel bath, cryogenic canes)

- Dilute the biological material before freezing procedure and after the thawing procedure (oocytes and embryos)
- Usage of special tanks with liquid nitrogen just for the biological samples which are frozen with open vitrification system in the time of pandemic. If the samples are frozen with the closed vitrification system, it is recommended to use the standard vitrification protocol.

### ***Management of patients positive for SARS-CoV-2 virus during the IVF procedure***

Considering the fact that there is still no scientific evidence on different pathways of transmission of the virus, as well as on the impact of the virus on human gametes and embryos, we are obliged to ensure that measures are taken to prevent and control the spread of infection among our patients as well as among healthcare personnel participating in the procedures of medically assisted reproduction.<sup>29, 30</sup> If the MAR centre does not meet the technical standards for safe epidemiological treatment prescribed in the treatment of COVID-19 positive patients<sup>31</sup> (which includes a separate aspiration room with an associated separate IVF laboratory for COVID-19 positive patients only), we suggest the following:

1. If symptoms occur and/or a patient tests positive for COVID-19 at an earlier stage of the procedure (before aspiration or embryo thawing): cancel and postpone the treatment.
2. If symptoms occur after aspiration, and during embryo cultivation, confirm the infection with a test: freeze all, with special care on safe cryopreservation.

If symptoms occur and we have a positive test before aspiration, but the patient is at high risk (life-threatening) of ovarian hyperstimulation (OHSS) or is an oncofertility patient, in those exceptional cases, aspiration of oocytes will be performed in a specially equipped and separate aspiration room intended for use with COVID-19 positive patients, where all epidemiological measures are respected: special procedure for putting on and removing PPE (e.g., personal protective equipment: isolation gowns and coveralls, surgical cap, surgical mask, goggles, face shield, two pairs of nitrile gloves), separate entrance and exit, ventilation area before undressing, tent for equipment removal.

After the aspiration, additional three hours are required in this area to cryopreserve the oocytes, but only if there is an associated separated IVF laboratory for COVID-19 positive patients (laminar flow hood with heated stage, stereo microscope, a CO<sub>2</sub> incubator with gas mixture supply, portable liquid nitrogen container, cooling rack).

Unfortunately, no IVF laboratory in Croatia meets the technical standards prescribed in the treatment of COVID-19-positive patients and we would not be able

to complete the procedure for such patients. The procedure of aspiration of oocytes in a specially equipped and separate aspiration room intended for use with COVID-19 positive patients requires a significant financial investment, but it is the only way to safely handle these conditions and quality storage of biological material that is extremely sensitive to external conditions (temperature, CO<sub>2</sub>, pH).

### ***Recommendation regarding laboratory work safety***

Guidelines for reducing the risk of SARS-CoV-2 transmission during medically assisted reproduction treatments in homologous procedures (non-donor fertilization):

- Planning MAR procedures exclusively with patients that have been proven healthy in centres that can adjust their work to the new circumstances.
- IVF laboratory preparation for restarting work includes cleaning and sterilization of incubators, cleaning of all equipment and working surfaces, walls, floors, doors and passages, as well as purchasing media and materials.
- Cleaning of the laboratory according to prescribed standard operative procedures (SOP), but with intensified frequency after every patient or work with a particular biological sample. Recommended intervals between two oocyte aspirations is a minimum of 1 hour needed for the adequate finishing of procedures for one couple and preparation for the next couple.
- After restarting work, all IVF laboratories need to work in the regime of high vigilance according to ESHRE21 and EDQM (European Directorate for the Quality of Medicines)<sup>32</sup> guidelines in order to obtain quality and safety working with tissues, cells and organs.
- Health workers need to wear masks, caps, gloves and safety gowns covering the skin.
- If there are two or more embryologists working in the laboratory, it is necessary to organize work in separate working hours; in other words, separation in two work teams (A and B) formed of a doctor gynecologist, a nurse and a clinical embryologist. In extreme cases when clinical embryologists need to go into quarantine/isolation, the patients are taken care of in a cooperative facility.
- Clinical embryologists need to minimize their contact with the rest of the working staff and patients. If they need to communicate it is important to keep a distance of 2 meters while wearing a mandatory protective face mask.

The handling of samples is based on guidelines without scientific evidence, but applied as precautionary measures:

- Semen samples for MAR procedures are collected at home and brought by men with minimal time spent in the IVF facility.

- Sperm preparation technique with density-gradient followed by swim-up technique<sup>14</sup>
- Additional oocyte washing after isolation from follicular fluid
- ICSI (Intracytoplasmic Sperm Injection) is recommended for fertilization
- Additional washing of embryos prior to embryo transfer in uterus
- Additional washing of embryos after embryo thawing

Considering the fact that there is still no scientific evidence on different pathways of transmission of the virus, as well as on the impact of the virus on human gametes and embryos, we are obliged to ensure that measures are taken to prevent and control the spread of infection among our patients as well as among healthcare personnel participating in the procedures of medically assisted reproduction. If the IVF centre does not meet the technical standards for safe epidemiological treatment prescribed in the treatment of Covid-19 positive patients (which includes a separate aspiration room with an associated IVF laboratory for Covid-19 positive patients only), we suggest the following:

1. If symptoms occur and / or patient tests positive for Covid-19 at an earlier stage of the procedure (before aspiration or embryo thawing): cancel and postpone the treatment.
2. If symptoms occur after aspiration, and during embryo cultivation, confirm the infection with a test: freeze all, with special care on safe cryopreservation.

## CONCLUSIONS

1. The triage of personnel and patients according to the questionnaire, with further COVID-19 testing based on the triage.
2. The method used for virus detection is RT-qPCR amplification.
3. Both partners that are entering the IVF/ICSI treatment need to be tested for COVID-19 two days prior to follicle aspiration. Male partners in the intrauterine insemination (IUI) procedure, as well as female patients undergoing the frozen embryo transfer (FET) procedure need to be tested two days before the procedures.
4. In cases of a positive COVID-19 test, the patients' treatment cycle is stopped.
5. In cases of ovarian hyperstimulation syndrome and proven COVID-19 infection, follicle aspiration is done exclusively for the purpose of protecting the patients' health and not for in vitro procedure. Therefore, the follicular fluid is not delivered to the IVF laboratory, instead it is taken care of and disposed as prescribed.
6. Considering it is still unclear how the infection can be transmitted, and testing may result in false negatives due to the inappropriate handling

of swab specimens, all epidemiological guidelines and the Croatian Society for Human Reproduction guidelines (Croatian Society of Clinical Embryologists - Guidelines on the health epidemiological framework for implementation in medically assisted reproduction procedures during COVID-19 pandemic regarding patients and medical health workers safety) are applied.

7. If a female patient expresses COVID-19 symptoms before the day of the embryo transfer, embryos are to be frozen in a closed type carrier according to standard procedure or stored in a separate cryotank.
8. Embryologists are working in a two-team regime, organized for rotational work every two weeks, or in morning/afternoon shifts. A one-hour interval between daily shifts is necessary for laboratory cleaning.

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